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LILAC BLIGHT IN THE UNITED STATES

BY

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LILAC BLIGHT IN THE UNITED STATES[1]

By MARY K. BRYAN

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INTRODUCTION

In the summer of 1925 some blighted lilac twigs were sent to the pathological laboratory of the Bureau of Plant Industry by H. W. Anderson, of Urbana, Ill., with the inquiry whether this was the bacterial disease described by Van Hall from the Netherlands and hitherto unreported in the United States. The twigs were blackened wholly or on one side only; cross sections showed the discoloration extending sometimes only through the cortex but often involving the entire thickness of the stem. The discolored tissues of the cortex swarmed with bacteria. Poured plates yielded practically only one type of colony. This was round, white, and caused green fluorescence in the agar, but its most striking characteristic was a wrinkling of the surface, which began on the second day and was persistent. As no mention was made of this very noticeable character by Van Hall (*2*) [2] or by E. F. Smith in his unpublished notes it seemed unlikely that this was the organism with which they had worked. Good infections, however, were obtained with subcultures from these wrinkled colonies. Since no culture of *Bacterium syringae* (Van Hall) EFS. (*Pseudomonas syringae* Van Hall) was available for comparison, cultural work was carried on and the cultures compared with the brief published and unpublished descriptions of *Bact. syringae*. The results were inconclusive, however, although there was no substantial disagreement aside from the colony character mentioned above.

In the spring of 1926, Westerdijk, of the Netherlands, sent the writer some blighted lilac twigs from which the causal bacterium was readily and repeatedly isolated. This organism was proved infectious by inoculations on lilac twigs. None of the colonies in any of the isolations and reisolations from this material showed any trace of wrinkling.

Cultural comparisons of the Netherlands and Illinois isolations were then made, but no real differences aside from that mentioned were found. The effects produced by inoculation with the two strains likewise showed no distinguishable differences. (Fig. 1, A and B.) It seems evident, therefore, that the two are strains of the same organism (*Bacterium syringae*) and that the Illinois disease is identical with the Netherlands disease.

While the work here reported was in progress a note appeared by C. O. Smith (*7*) in which he states that *Bacterium citriputeale*, the cause of citrus blast and black pit, is also probably responsible for a new lilac blight in California. To quote: "This investigation is

[1] Received for publication Oct. 3, 1927; issued April, 1928.

[2] Reference is made by number (italic) to "Literature cited," p. 235.

well in progress, and the results thus far indicate that the citrus blast, the avocado blemish, and the California lilac disease are closely related and probably are caused by the same organism." Com-

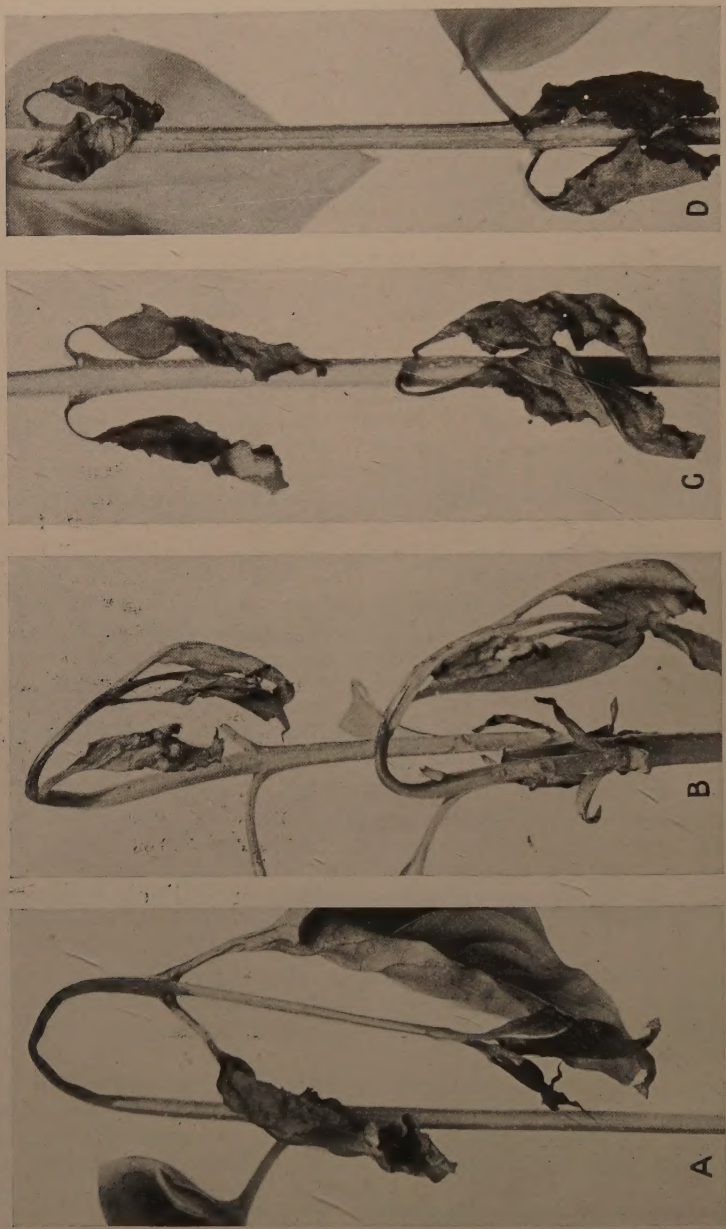


FIG. 1.—A, Lilac twig 12 days after prick inoculation with *Bacterium syringae* from the Netherlands; B, lilac twig 10 days after prick inoculation with *Bact. syringae* from Illinois; C, lilac leaves inoculated in the petioles with *Bact. syringae* from Illinois; D, lilac leaves inoculated in the petioles with *Bact. citriputeale*

parisons were therefore made between *Bact. syringae* isolated from Illinois and Netherlands lilacs with a freshly isolated culture of *Bact. citriputeale* received from Fawcett in California. Parallel cultures proved the latter to be identical with *Bact. syringae* except

in colony characters on agar, and the differences in these were no more striking than the differences between the Netherlands and the Illinois strains. Inoculations on lilac with *Bact. citriputeale* gave good typical infections. (Fig. 1, D.) Good black-pit lesions were also produced on lemons by needle pricks with both the Netherlands and the Illinois strain; but these strains were somewhat less virulent on lemons than *Bact. citriputeale.*

From these comparisons it seems probable that the lilac blight of California is identical with that in Illinois and the Netherlands.

HISTORY OF THE DISEASE

Lilac blight has heretofore been reported only from the Netherlands, Germany, and England.

It was first observed by Sorauer (*9*) in Germany in 1891. In 1899 Ritzema Bos (*5*) found it in the Netherlands. Both considered it to be bacterial. Beyerinck subsequently obtained infections on lilacs with an organism which he isolated and studied. He did not publish, but turned over the organism and the results of his inoculations to Van Hall (*2*) who in 1902 published a detailed account of the appearance and progress of the disease. Van Hall made cultural studies of the bacterium, which he named *Pseudomonas syringae,* although he did not succeed in producing any infections with his organism.

Güssow (*1*) in 1908 published his observations on a lilac blight in England which he ascribed to *Pseudomonas syringae.*

In 1906 E. F. Smith while in the Netherlands studied this blight, made observations on its effect on the plant, and obtained good infections with the bacterium which he isolated, confirming Van Hall's work. He did not, however, publish the results of his studies.

APPEARANCE OF DISEASED PLANTS

As described by Van Hall (*2*), Sorauer (*9*), and E. F. Smith, the disease in Europe is identical in appearance and development with that in Illinois. Güssow's (*1*) description of the blight in England does not, however, conform to these descriptions. He does not mention stem or petiole infections, but confines his account to the appearance of the leaf blades, and even here the symptoms are unlike those of the Netherlands disease. His reported infections on leaf blades from needle pricks did not appear until two months after inoculation, while *Bacterium syringae* gives evidence of infection by the third day. It seems quite improbable, therefore, that he was dealing with the lilac blight of the Netherlands.

On the specimens received from the Netherlands in 1926 there were very distinct dark water-soaked angular spots on some leaves, usually on those with infected petioles. These spots swarmed with bacteria and yielded pure cultures of *Bacterium syringae* on plating. There were also lesions on the peduncles and pedicels of the flower clusters, and many flower buds were completely blackened.

In 1925, when Anderson first observed the disease in a large planting of lilacs in an arboretum in Illinois, their appearance suggested pear blight. Entire young shoots were blackened, some were bent, others erect. In some cases only one side of the shoot was involved, a discolored streak from several millimeters to one-half the circum-

ference of the stem in width extending the entire length of the season's growth. No definite leaf spots were observed, but leaves on the infected parts invariably were partially or entirely blackened.

In 1926 the disease did not appear at all in the planting of lilacs where it was found the previous year (see "Control," p. 232), but a less virulent outbreak occurred in the same arboretum on young nursery stock at a considerable distance from this planting. The season had been very dry, and when the plants were observed by the writer in July only small stem lesions from 5 to 15 mm. long could be found. Above and below these lesions, however, the leaves were rather thickly peppered with small irregular to round brown spots surrounded by a yellowish halo. (Fig. 2, A.) On the leaves nearest the stem lesion the leaf blades were ragged with large brown areas, due to coalescing spots and the torsion caused by irregular development of healthy and diseased areas. (Fig. 2, A.) The smaller spots were identical in appearance with those produced by inoculation in Washington. (Fig. 2, B and C.) (See "Inoculations.")

The disease attacks the young shoots as they develop in early spring, the first symptoms being brown spots on leaves and internodes. These become black and increase rapidly in size, especially in rainy weather. Further development depends largely on the age of the part attacked. On somewhat mature stems the spots usually enlarge longitudinally, passing over to the petioles in their path, causing the death of these leaves, but not of the remainder of the twig, which remains upright. When the spots occur on very immature internodes, on the other hand, the infection spreads around the twig, girdling it completely for a distance of several centimeters. (Fig. 1, A and B.) The stem bends over at this lesion, and the part above it withers and dies.

Similar differences are observable in the results of infection on immature and mature leaves. The immature leaves blacken rapidly and completely, while on older ones the spots enlarge slowly and at the worst, by coalescing, blacken and kill large areas of the blade, causing distortion.

TISSUES INVOLVED

The disease is primarily one of the parenchyma, although the vascular region may become involved. In the stem the bacteria spread through the intercellular spaces of the cortex, causing blackening and collapse of the cells and frequently cavities of greater or less extent. If the infection reaches the vascular system it may run up through this channel without external lesions, evidencing itself first by a wilting of the upper leaves, later by sunken blackened areas on the stem along the line of internal infection. In such cases, cavities filled with bacteria are formed in the vascular region. In infected petioles the parenchyma is very susceptible. The whole thickness of the petiole blackens; the bacteria make their way into the vascular system and are to be found far up in the midrib and veins, while the leaf blade blackens.

INOCULATIONS

Prick inoculations were made in the tender upper part of young lilac suckers in August, 1925, using subcultures from colonies isolated from the Illinois lilac twigs. In three days the stem near the pricks

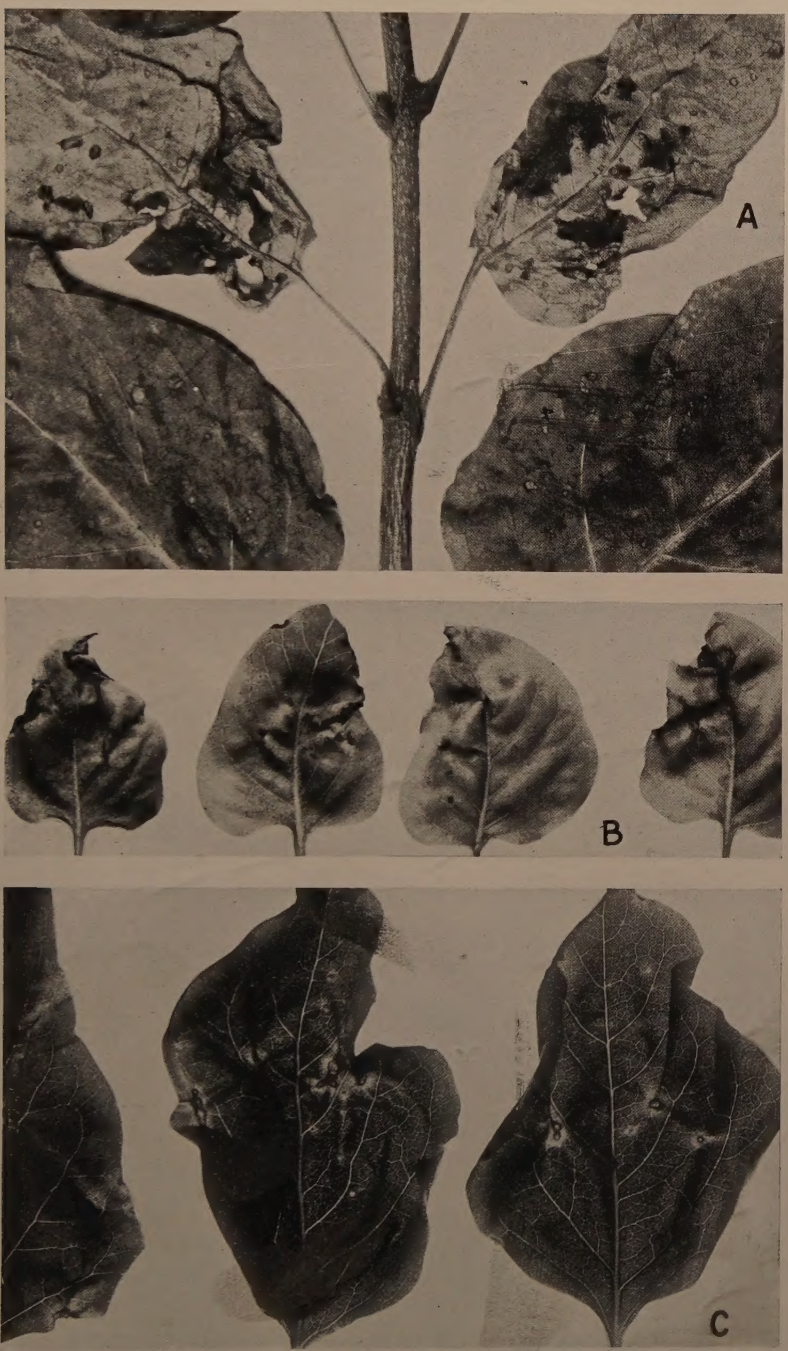


FIG. 2.—A, Natural infection on lilac leaves from Illinois, a small canker on the stem below the most seriously spotted leaves; B, lilac leaves 12 days after spraying with *Bacterium syringae* from Illinois; C, lilac leaves 14 days after spraying with *Bact. syringae* from Illinois

was blackened and the darkened area extended up into adjacent petioles. On the fourth day most of the inoculated shoots were completely dead and black above the point of inoculation. (Fig. 1, B.)

FIG. 3.—A, Infected flower clusters (the blackened drooping ones) inoculated by rubbing with *Bacterium syringae* from Illinois; B, lilac twig inoculated at X by needle pricks with Illinois strain of *Bact. syringae;* time, 16 days. The infection is advancing in the vascular region, showing in the surface lesions in two places and in the dying margins of the leaves

Isolations were made from one of these infections, and on August 25 subcultures were used to inoculate lilac shoots. Again infection was evident in two days, and after five days a dark streak extended downward through one or two internodes, invading the petioles in its

path but not causing a general blackening. Whether the whole twig blackened, or only a streak, seemed to depend largely on the age of the tissue inoculated. Pricks in the very tender tip always killed the twig; pricks made in slightly more mature wood gave only streaks. Sometimes infection spread in the cortex; in other cases it reached the vascular region quickly and spread rapidly up the stem through the vessels, as shown by the wilting of leaves in its path. Only later did a sunken black streak form externally along the line of internal infection. (Fig. 3, B.) Petioles were very susceptible, but infection did not often run back into the stem from them. When petioles became infected the whole leaf blade blackened, sometimes suddenly, at other times gradually.

The organism from the Netherlands, when used to inoculate lilacs in the pathological greenhouse, gave results identical with those obtained when the Illinois organism was used, except that the Netherlands organism seemed slightly more virulent. (Fig. 1, A.)

Stomatal infections on leaves were obtained by spraying and by rubbing with the Illinois organism and also by rubbing with the Netherlands isolations. Spots were round to irregular, brown, with a yellowish halo 1 or 2 mm. in width. The first evidence of infection was visible in five days, but the spots enlarged very slowly, causing slight distortion, and did not attain any considerable size under the very dry conditions prevailing during the lilac growing season. There was never any general spread of the disease from such infections. However, in the late fall of 1926, when rub inoculations were made on very tender leaves of young suckers during a period of rainy weather, infection spread so rapidly that individual stomatal infections were not observed. Leaves and petioles were rapidly and completely blackened. On most inoculated suckers there were also stem leasions 10 to 15 mm. long, black and sunken, and not continuous with diseased petioles, but apparently secondary stomatal infections. Isolations from these stem lesions gave wrinkled colonies from the stems inoculated with the Illinois organism and smooth colonies from those inoculated with the Netherlands organism.

SUSCEPTIBLE VARIETIES

The first observation of the disease in Illinois was on *Syringa vulgaris* only. In the nursery, however, it occurred on both purple and white sorts, *S. vulgaris* and the following varieties showing infection: Mont Blanc, Reine Elizabeth, Princess Alexandra, and Roi Albert.

Sorauer (*9*) reported that *Syringa chinensis* was less susceptible than other sorts, and Ritzema Bos (*5*) observed that *S. vulgaris* was more susceptible than *S. persica*.

ORIGIN OF THE DISEASE

An attempt to trace the infections in Illinois to importations failed, although there can be little doubt that the infection has at some time come to this country from Europe on nursery stock. Scions for the nursery where it was found came from several widely separated sources in the United States. No importations for this arboretum had been made from Europe except a few plants from France which had never been near either of the places in the arbore-

tum where infections were found, and none of the French importations showed the disease. Moreover, the disease is not known to exist in France.

CONTROL

From the nature of the disease it is evident that sprays would be of little value and that destruction of diseased shoots is the only practical remedy. Careful cutting out was used in Illinois where the disease was severe in 1925, and there was no recurrence in 1926. The unusually dry season, however, may have been a factor in the completeness of its disappearance, since the disease elsewhere was very mild in its effects.

THE ORGANISM

CULTURAL CHARACTERS

Unless otherwise stated, all beef media used were made with beef infusion and had a P_H of 6.8 to 7.2.

AGAR PLATES.—On peptone beef-infusion agar, colonies of *Bacterium syringae* Netherlands are visible in 24 to 48 hours. These are round, white, smooth, convex, finely crosshatched, and opalescent by oblique transmitted light, transparent, attaining in six or seven days a width of 5 mm. and becoming flat, but otherwise retaining all of the characters mentioned above. There is no surface wrinkling at any stage. (Pl. 1, B, D, F, and G.) The agar becomes green.

Colonies of *Bacterium syringae* Illinois, on the same agar are visible in 24 to 48 hours and at this time are indistinguishable from colonies of the Netherlands strain. On the day after they appear the center has become sunken and holds one to several clear droplets. (Pl. 1, C.) By the third day the center is deeply wrinkled, these wrinkles increasing with the growth of the colony, while the margin remains raised, smooth, and finely crosshatched. Colonies may attain a diameter of 5 mm. by the sixth and seventh day. (Pl. 1, E and H.) The agar becomes green on all beef-infusion media.

Very occasionally in direct isolations of the Illinois organism, but frequently in plates from cultures, a very few smooth colonies appear; that is, some colonies retain the smooth surface of the first day and never show any wrinkling. These when used for further plating usually give only smooth colonies, but occasionally a few wrinkled ones appear.

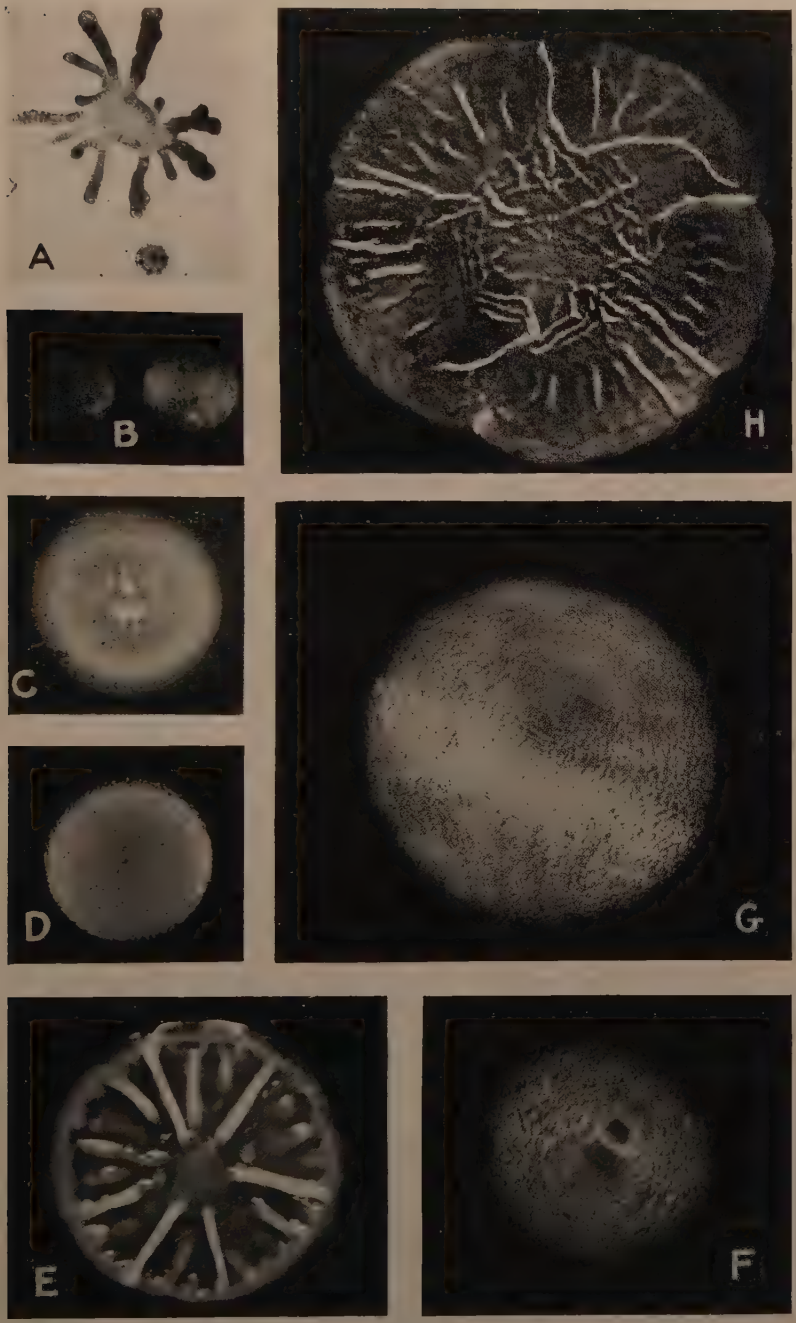
Rough and smooth strains of the same organism seem to be common to many organisms. The interconvertibility of the two types has recently been reported by Jordan (*3*) for certain animal pathogenes and by Sharp (*6*) for *Bacterium phaseoli sojense*. Rough colonies are not unusual in plates of smooth-colonied plant pathogenes. In this case the preponderance in the number of rough colonies is the unusual factor.

Occasionally spreading fimbriate colonies appeared on plates of the Illinois organism (pl. 2, A), never on those of the Netherlands organism. These colonies occasionally remained smooth but mostly became wrinkled. (Pl. 1, A.) Their occurrence seemed to depend on the surface tension of the agar, for they generally appeared on thicker portions of agar, due to an uneven bottom in the plate, and could be induced by using 20 c. c. instead of 10 c. c. of agar for a plate. This could not be done with the Netherlands strain.

Occasionally colonies very irregular in outline, slightly roughened on the surface, and very coarsely crosshatched appeared with both the Netherlands and the Illinois isolations in platings from pure cultures. This form was very stable and has never been observed to revert to either of the other forms. It was very weakly pathogenic.

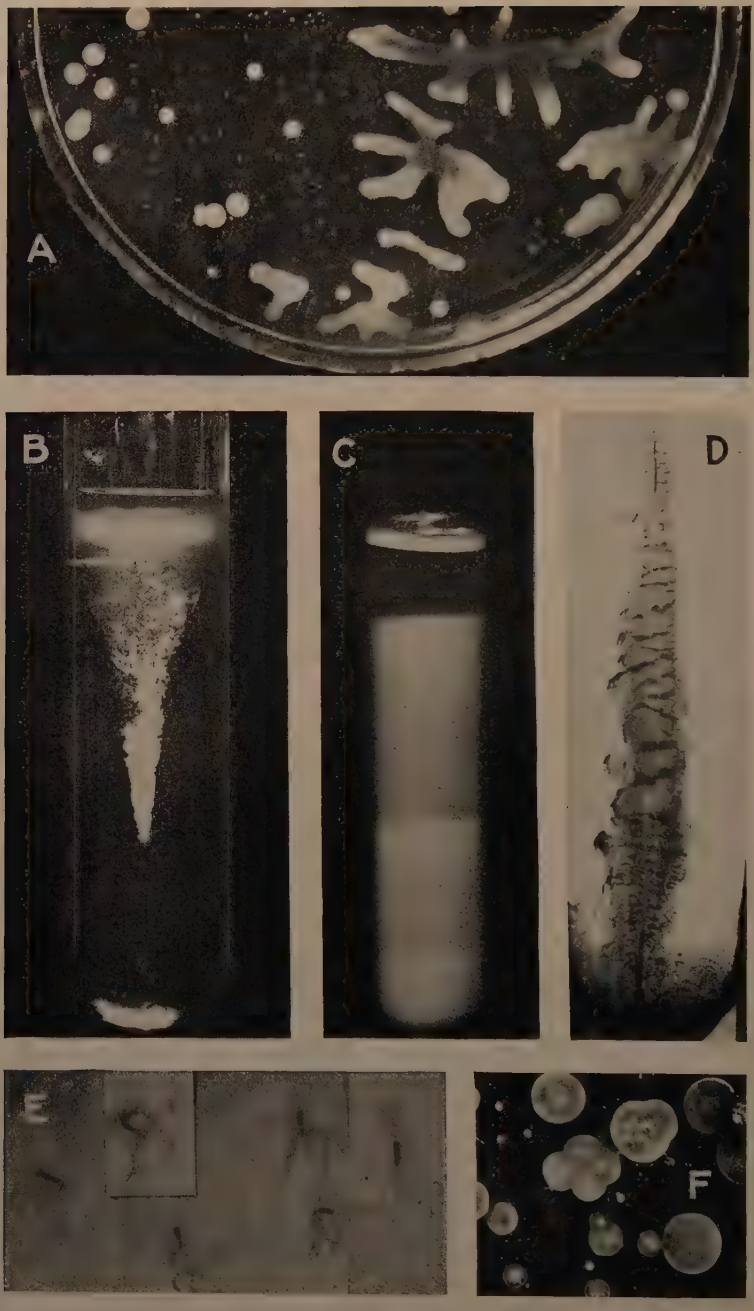
BEEF-AGAR SLANTS.—The same difference in surface in the two strains was apparent on beef-agar slants as on plates. (Pl. 2, D.) Growth is 3 to 7 mm. wide, white, transparent, opalescent, finely crosshatched by oblique light, with wavy or scalloped margins. The whole of the agar becomes green. Small crystals form in abundance just below the growth on the slant and below the V.

BEEF-AGAR STABS.—Growth characteristic of each strain occurs on the surface, finally covering it. In the stab, scanty growth is visible for less than one-third the length of the needle track. Small crystals form just below the surface of the agar.



Colonies of *Bacterium syringae* on beef-infusion agar

A. Fimbriate colony, 4 days old, natural size
B. Illinois strain, 24 hours old. Colonies of the Holland strain are indistinguishable from these at this age
C. Illinois strain, 2 days old
D. Holland strain 2 days old
E. Illinois strain, 3 days old
F. Holland strain, 3 days old
G. Holland strain, 5 days old
H. Illinois strain, 5 days old
(B to H, × 10)



A. Round and fimbriate colonies on beef-infusion agar plate, 2 days old
B. Gelatin stab, 48 hours old at 18° C.
C. Litmus milk culture, 4 days old
D. Beef-infusion agar slant, 3 days old
E. *Bacterium syringae* showing polar flagella
F. Colonies on beef-infusion gelatin plate, 2 days old

BEEF BROTH.—In peptone beef-infusion broth clouding is evident within 24 hours. Green fluorescence begins at the top on the second day, and a delicate filmy shining wrinkled pellicle forms which breaks into fragments readily on shaking. This pellicle is characteristic of wrinkled colonies only. Other types of colony have a dull white opaque pellicle which disappears as a fine cloud on shaking. All types produce the green fluorescence. Numerous small crystals lie in the white precipitate.

BEEF-EXTRACT MEDIA.—Growth is less vigorous on beef-extract agar than on beef-infusion agar, colonies attaining only approximately one-half the size under the same conditions for growth. No surface wrinkling occurs and no green fluorescence develops on plates, slants, or in broth made with beef extract, and only occasional small crystals are formed.

POTATO CYLINDERS.—On steamed potato cylinders growth is thin, grayish white, never becoming abundant. The potato is browned.

COHN'S SOLUTION.—There is very weak growth in Cohn's solution, evidenced by a faint but decided clouding and a very small quantity of white precipitate.

USCHINSKY'S SOLUTION.—Clouding is moderate to heavy in this medium, with a delicate rim and pellicle and a very pronounced green color. An abundant white precipitate is formed. No crystals were observed. The Netherlands strain gave heavier growth than the Illinois strain, as evidenced by both clouding and pellicle.

FERMI'S SOLUTION.—In Fermi's solution a heavy pellicle is formed, but the fluid is only moderately clouded and takes on a beautiful blue-green fluorescence.

BLOOD SERUM.—There is moderate growth on blood serum, but no liquefaction takes place.

PHYSIOLOGICAL CHARACTERS

LIQUEFACTION OF GELATIN.—In beef-peptone-gelatin stabs, made with both beef extract and beef infusion, liquefaction begins within 24 hours at 18° C. and is infundibuliform. Within a day or two it becomes stratiform at the top with a narrow funnel of liquefaction reaching one-half the depth of the gelatin or more and largely filled with growth. (Pl. 2, B.) When inoculations are made from freshly isolated cultures, liquefaction is rapid, often complete in 6 days, but when old isolations are used liquefaction progresses much more slowly and occasionally never is really complete, although the start is prompt and infundibuliform. The surface layer is green fluorescent. There is complete liquefaction and green fluorescence throughout in 6 to 16 days. With cultures which had been out of the plant for months, liquefaction began promptly at 18°, but progressed much more slowly and sometimes was not complete after six weeks.

FERMENTATION OF SUGARS.—Fermentation was tested on beef-extract agar and synthetic (ammonium phosphate) agar containing brom cresol purple with saccharose, dextrose, maltose, lactose, galactose, levulose, glycerin, and mannit. Acid was produced, beginning on the second day, from all except lactose and maltose, the agar becoming bright yellow throughout. Glycerin and mannit were somewhat less acid than the others. Cultures were held for two weeks. In the beef-extract agar, but not in the synthetic agar, alkali production began in five days and extended through two-thirds of the culture in saccharose, dextrose, galactose, and levulose. The two strains were alike with all the sugars tested.

In fermentation tubes containing 1 per cent peptone water with the same sugars there was no gas. Clouding appeared in the closed end with saccharose and dextrose only, heavier in saccharose in both strains.

RELATION TO FREE OXYGEN.—The organism is not strictly aerobic, since growth takes place in the closed end of fermentation tubes with saccharose and dextrose. In stab cultures, however, there is only scanty growth about one-third the depth of the needle track even when the sugars mentioned above are present.

HYDROLYSIS OF STARCH.—There is very slight diastatic action. Starch-agar plates seven days old, when flooded with iodine solution, show a purple area 3 to 8 mm. wide around the growth, while on potato cylinders growth is scanty.

NITRATE REDUCTION.—There is no nitrite reaction when 10-day-old nitrate beef-broth cultures are tested with the starch-potassium iodide-sulphuric acid method, but a very decided though very moderate nitrite reaction when the α-naphthalymine-sulphanilic acid test was used. This would indicate very weak nitrate reduction.

ACID IN MILK.—No acid is formed in milk, but a slight alkalinity is evident in litmus milk.

REDUCTION OF LITMUS.—Litmus milk is blued slightly. A layer of clear whey 3 to 5 mm. forms at the top, but there is no further evidence of coagulation. Peptonization then begins and progresses downward in bands (pl. 2, C), followed by reduction of the litmus. Peptonization and reduction are complete within two weeks. Within the next two weeks the color returns to a dark grayish blue and remains so indefinitely.

REDUCTION OF METHYLENE BLUE.—Methylene blue in milk is reduced promptly and completely, the reduction beginning by the second day and becoming complete on the third or fourth day. By the fourth week the color returns a clear green (forest green according to Ridgway (*4*)).

AMMONIA PRODUCTION.—Ammonia production is strong in beef-peptone media.

HYDROGEN SULPHIDE.—Hydrogen sulphide is produced very scantily or not at all.

PRODUCTION OF INDOL.—No indol was produced in Dunham's solution within 20 days, tests being made at intervals of 5 days.

TOLERATION OF ACID.—Tests were made in beef-infusion broth and in beef-extract broth with a series of P_H values. Growth occurred in beef-infusion broth from P_H 5.4 to P_H 9.1, with the optimum at P_H 6.8, and in extract broth from P_H 5.3 to 9, with the optimum at P_H 6.5 to 7.

TOLERATION OF SODIUM CHLORIDE.—There is clouding within 24 hours in beef broth containing 1, 2, and 3 per cent NaCl, in 2 days in 4 per cent, and after 5 days in 5 per cent. Very slight growth takes place in 6 per cent. In 3 to 6 per cent NaCl growth consists of distorted irregularly swollen filaments.

RESISTANCE TO DESICCATION.—The organism is very sensitive to drying. Drops of a 24-hour-old culture placed on sterile cover glasses in sterile Petri dishes and kept in the dark gave no clouding when dropped into beef broth after 2 days' drying.

TEMPERATURE RELATIONS.—Best growth occurs at 28° to 30° C. Beef-broth cultures kept at 35°, 36°, and 37° for 8 days remained clear. When removed to room temperature (28° C.) the 35° cultures clouded but the 36° and 37° cultures remained clear. Within 24 hours clouding was visible in beef-broth cultures at temperatures from 10° to 30° and after 5 days at 1° to 2.5°. The thermal death point is 51° for both strains.

BRIEF CHARACTERIZATION OF BACTERIUM SYRINGAE[3]

Bacterium syringae is a motile rod 1.2 to 1.8 by 0.6 μ with one to several polar flagella; single, in pairs or short chains. Long twisted filaments and chains occur in salt bouillon. No spores are formed. A small capsule may be demonstrated by Ribbert's dahlia stain. The organism is Gram-negative, but stains readily with the ordinary bacterial stains. An irregular or polar staining is obtained with carbol fuchsine. Agar colonies round or fimbriate, smooth or wrinkled, white; liquefies gelatin rapidly; produces green fluorescence in beef-infusion media; reduces litmus and methylene blue in milk; forms a soft rennet curd in milk and clears it in bands; produces ammonia but no indol or hydrogen sulphide; weak nitrate reduction; feeble diastatic action on starch; grows well in Uschinsky's solution and Fermi's solution, but very feebly in Cohn's solution; produces acid from saccharose, dextrose, galactose, levulose, glycerin, and mannit, but not from lactose or maltose within two weeks; no gas; tolerates 5 per cent NaCl in beef broth; optimum temperature 28° to 30° C., maximum 35°, minimum below 1°.

SUMMARY

A lilac blight occurring in Illinois has been proved to be identical with the lilac blight of Europe caused by *Bacterium syringae* (Van Hall) EFS.

The disease blackens and kills young stems and leaves in the spring. It is most destructive in rainy seasons. The appearance of affected plants suggests fire blight.

The blight is primarily a disease of the parenchyma, but it also invades the vascular tissue.

Careful pruning out of diseased twigs is the best method of control.

Cultural characters of the organism are given.

[3] "Bacterium" is here used according to the system of nomenclature proposed by Erwin F. Smith (*8, p. 711*) and used by him for this organism in the same volume (*8, p. 65*).

Bacterium citriputeale has been found indistinguishable from *Bact. syringae* in cultures, and cross inoculations with the two organisms have produced good infections.

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